

We claim:

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1. A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

- 10 a) inputting an ensemble of protein backbone scaffolds;
b) applying at least one protein design cycle to each of said scaffolds; and
c) generating a probability matrix derived from a plurality of variable sequences.

15 2. A method according to claim 1 wherein said protein design cycle comprises a sequence prediction algorithm.

20 3. A method according to claim 1 wherein said protein design cycle comprises a dead end elimination algorithm.

4. A method according to claim 1 wherein said protein design cycle comprises a genetic algorithm.

25 5. A method according to claim 1 wherein said protein design cycle comprises a Monte Carlo algorithm.

6. A method according to claim 1 wherein said protein design cycle comprises a self consistent mean field theory (SCMF) algorithm.

30 7. A method according to claim 1 wherein said protein design cycle comprises a Monte Carlo algorithm.

8. A method according to claim 1 further comprising ranking said variable sequences.
- 5 9. A method according to claim 1 further comprising synthesizing a plurality of said variable sequences.
- 10 10. A method according to claim 1 further comprising recombining a plurality of said variable sequences to form additional variable sequences.
11. A method according to claim 1 wherein said ensemble comprises a family of naturally occurring proteins.
- 15 12. A method according to claim 1 wherein said ensemble is generated by a Monte Carlo simulation.
13. A method according to claim 1 wherein said ensemble is generated by a Molecular Dynamics simulation.
- 20 14. A method according to claim 1 wherein said ensemble is derived from an NMR ensemble of structures.
15. A method according to claim 1 wherein after step a), a rotamer library is pre-filtered to eliminate high energy interactions to form a suitable rotamer set for each scaffold.
- 25 16. A method for optimizing simulation or scoring function parameters that utilizes comparisons between designed sequences and natural sequences, comprising the steps of:
 - 30 a) applying a protein design cycle to produce a variable protein sequence;
 - b) comparing said variable protein sequence to at least one natural protein

sequence and/or conformation;

c) modifying said simulation or scoring function parameters to reflect said comparison.

5 17. A method for optimizing simulation or scoring function parameters that utilizes comparisons between designed sequences and natural sequences, comprising the steps of:

a) applying a protein design cycle to produce an amino acid probability matrix;

10 b) comparing said matrix to at least one natural protein sequence and/or conformation;

c) modifying said simulation or scoring function parameters to reflect said comparison.

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